International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:5, No:11, 2011

The Presence of Enterobacters (E.Coli and Salmonella spp.) in Industrial Growing Poultry in Albania

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***Abstract*—**The development of the poultry industry in Albania is mainly based on the existence of intensive modern farms with huge capacities, which often are mixed with other forms. Colibacillosis is commonly displayed regardless of the type of breeding, delivering high mortality in poultry industry. The mechanisms with which pathogen enterobacters are able to cause the infection in poultry are not yet clear. The routine diagnose in the field, followed by isolation of *E. coli* and species of Salmonella genres in reference laboratories cannot lead in classification or full recognition of circulative strains in a territory, if it is not performed a differentiation among the present microorganisms in intensive farms and those in rural areas. In this study were isolated 1.496 strains of *E. coli and* 378 *Salmonella spp.* This study, presents distribution of poultry pathogenosity of *E.coli and Salmonella spp.,* based on the usage of innovative diagnostic methods.

***Keywords*—**poultry, E.coli, Salmonella spp., Enterobacter

I. Introduction

COLIBACILLOSIS and Salmonella are acute and chronic diseases of poultry, with clinical outbreaks in chickens and turkeys. In the poultry industry, colibacillosis are displayed regardless of the type of breeding (rural or intensive), delivering high morbidity and mortality in flocks, and therefore significant economic loss. Bacteria of E. *coli* and Salmonella spp. normally colonize the digestive tract of mammals and poultry [1]. In most mammals colibacillosis is one of the main enteric diseases, while in poultry it is mainly an extra intestinal and systemic disease, with the outbreaks after breaking the host defensive barriers from other primary diseases or as a result of the presence of virulent strains of E. *coli* residing in the micro intestinal flora of macro organism, [2]. Worldwide, for the control of many bacterial diseases in poultry industry, prevention and treatment doses of antibiotics are commonly used, along with administration of their food ration and / or drinking water. It was noted that this practice has a positive effect on growth of the additional weight and food conversion. Empirical antibiotic management agents in poultry, has exerted a selective pressure, which in itself explains the phenomenon of antibiotic resistance, encountered in a large number of resident bacteria in the organism of birds [3].The antibiotic resistance comes as a result of complex and multifactorial process, which relies on the involvement of cellular genetic elements, which are carriers of the resistance transfer factors [4]. Acquisition of R plasmid codifying genes is due to the exchange of genetic material from one bacterium to another. Some R plasmid may also carry other virulence factors as well, such as bacteriocins, siderofors, citotoxins and adherence factors [5].The inappropriate use of Fluoroquinolone in poultry breeding industry promotes the appearance of a cross resistance to the drug used in the treatment of enteric infections in humans [6]. Also, many studies take into consideration the numerous cases of the cross-antibiotic resistance towards the Tetracycline group (Chlortetracyclina, Oxytetracyclina and Tetracycline) in animals and poultry breeding to produce products with animal and human origin [7].Bibliographic sources present an obvious increase in the occurrence of poultry antibiotic resistance, as a result of uncontrolled use of antimicrobial agents both during drug treatment of many bacterial infections, as well as their use as additives in food rations [8].Moreover, this microbial resistance is similar to E. *coli* isolated from people who have direct contact with these birds. Such strains are seen to be similar in the possession and expression of virulence factors in humans, as well as in birds. These data provide evidence for possible transmission of resistant microorganisms or plasmids, from poultry to people [9], [10].

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II. Materials And Method

In the time frame of 2005 - 2009, by poultry farms located in different geographical areas within the territory of Albania (Fier, Kavaje, Durres, Elbasan, Shkoder, Korce, Lezhe dhe Lushnje), were selected visceral organs and intestinal materials.

The materials were randomly selected and it was based on the clinical outbreak cases of colibacilliosis and salmonellas and sporadic reports of infections screening by Bacterologic Laboratory, of Food Safety and Veterinary Institute (I.S.U.V) in Albania.

The pathological material taken from chicken carcasses (dead birds) was used for isolation of E. coli and Salmonella spp. Firstly, the material was taken by burning the organ's surface to prevent them from mixing with banal flora, and then planted was carried out in the culture plates and differentiation terrains, such as: broth, Endo and McConkey. The planted terrains were placed to be incubated in thermostat with temperature 37 ° C for a period of 24 to 48 hours. Then, the planted cultures were checked out after a 24 hours of the incubation period.

For E. *coli*, the differentiating Endo terrains will grew the average colony of red shiny metal (the terrain acidification, lactose positive); while in McConkey will grow pink colonies, colonies that are of type S (Figure 1). While, in microscopic layouts with Gram method will be appeared average gram negative rods which are uniform in size (Figure 2). In order to separate bacterium E. coli from broth cultures, it was transferred in Gasnar and XLD selective terrains, and placed for cultivation in thermostat at 37 degrees C for 24 hours.

A typical coliform colony based on morphological characteristics (lactose - positive) through a sterile needle is transferred in a sterile test tube, containing 10 ml broth and placed for incubation at 37^0 C for 24 hours. After incubation, the indole test was carried out by dropping one (1) drop of Erlih solution in the test tube (epruveta walls) filled with broth culture (24 hrs). In positive cases, a red ring creation was created, on the broth culture surfaces. By selective DC terrain through a sterile needle a colony for the each culture of E. coli was taken and transferred to 10 ml broth Brilliant Green Bile 2% (OXOID), where a Durham bell was previously reversed. The test is considered positive if after 24 hours incubation, was noticed the presence of gas inside the Durham bell. For the characterization of E. *coli*., the enterotube or API 20E system was used.

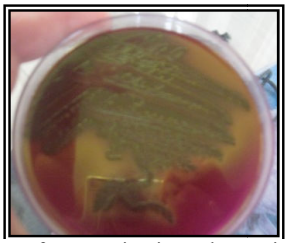

Fig. 1 Lactose fermentation in Endo terrain by E.Coli

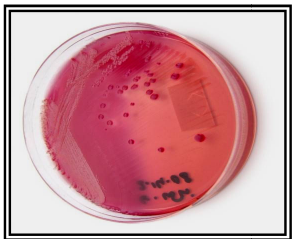

Fig. 2 Rose colonies in agar McConkey tipical for E.coli

For Salmonella spp., initially was preceded with the burning of surface organs taken from carcasses with a spatula and then their surface cutting was carried out with scissors in the cube form. The cutting pieces were inoculated in growth and differentiating endo, blood agar and broth terrains and then placed to be incubated in a thermostat at 37 ° C for 18 - 24 hours. After incubation in broth, a diffuse increase was observed: in blood agar was shown small grey shiny colonies, which were of type S; while in differentiating Endo terrain, salmonella colonies were small, smooth and with color of the respective terrain. Agar Mc.Conkey is inhibitory terrain for non enteric microorganisms. Their cultivation in this terrain made possible the differentiation of microorganisms that ferment the lactoze by microorganisms that do not ferment the lactoze. The II-nd phase had to do with transferring of an amount culture taken from a 24 hrs broth culture in selective terrains. SS terrain was inhibitory for non enteric microorganisms. In this terrain will grew only salmonella colonies, which were small, colorless, smooth and with black centers respectively. To identify the casual the API 20 system was used, where the reading of biochemical reactions that occurs in API 20 system was made through respective coding manuals that follow the kits.

III. DISCUSSIONS

For the purpose of this study, a total of 1.496 *E. Coli* and 378 Salmonella spp., strains were isolated during the period of 2006 to 2010.

All of 1.496 E. *Coli* and 378 Salmonella spp. obtained strains were diferentiated according to years and the presence of E. *Coli*, Salmonella spp. in the izolates which were divided acording to grop's age. Chart 1 presents the results of obtained strains which breakdown by years of study period:

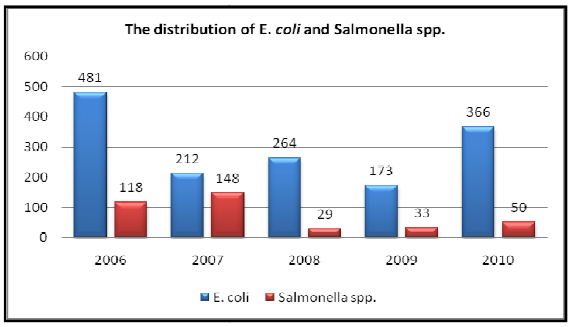

Chart 1. the distribution of E. coli and Salmonella spp. isolates across the study period

The izolates of E. *coli* (1.496) and those from Salmonella spp. (378) were grouped acorging to the age group and their obtained source. Therefore, the table 1 shows the number of isolated strains in chicken eggs, broilers, turkeys and ostrichs. It is important to note that the number of obtained isolates was higher in matured poultry, emphasing the fact that the poultry lifespan is related with the presence of many infection sources.

TABLE I
THE PRESENCE OF E. COLI, SALMONELLA SPP. ACCORDING TO GROUP – AGE

Strain characteristics	**E. *coli***		**Salmonella. spp**	
	Chicken /birds	**Mature**	**Chicken/ bird**	**Mature**
Chicken eggs	198	507	113	208
Broiler	297	360	0	15
Turkey	117	13	29	13
Ostrich	0	4	0	0

In order to facilitate the study results, E. *coli* and Salmonella spp. isolates are grouped according to their production sort: chicken for eggs or broilers. The aim of this

differentiation is to help us for other study objectives, especially those related with antibiotico resistance. Table II demonstrates the distribution of E. *coli and* Salmonella spp. isolated during the 2006 – 2010 periods. All of E. *coli* strains were isolated from chicken carcasses by colibacilliosis or with similar clinical signs of this infection, as well as from other poultries carrion from other viral infections. While 378 Salmonella spp., strains were also isolated from poultries with clinical signs of salmonella infection or from poultries carrion from other infections.

TABLE II
THE PRESENCE OF E. COLI AND SALMONELLA SPP. ISOLATES ACCORDING TO THEIR PRODUCTION TYPE

Strain characteristics	E. *coli*	%	Salmonella spp	%
Chicken eggs	705	47.1	321	84.9
Broiler	657	43.9	15	3.96
Turkey	130	8.68	42	11.2
Ostrich	4	0.26	0	-
TOTAL	1.496	100	378	100

The obtained E. *coli* Salmonella strains were differentiated according to their group-age and production type, which are summarised as follow:

- 705 (47.1%) isolates of E. *coli* strains from the chicken eggs belong to the group- age: chicken/birds and mature;
- 675 (43.9%) isolates of E. *coli* strains from broilers belong to the group- age: chicken/birds and mature birds;
- 208 (84.9%) isolates of Salmonella spp. from chicken eggs belong to the group- age: chicken/birds and mature birds;
- 15 (3.96%) isolates of Salmonella spp. from broiler belong to the group- age: chicken/birds and mature birds;
- 130 (8.68%) strains of E. *coli* and 42 (11.2%) strains of Salmonella spp. were obtained from turkey;
- Only a small number of E. *coli* strains (0.26) were isolated from ostrichs.

In conclusion, the E. *coli* and Salmonella spp. strains, isolated from analysed poultry in this study, showed morphological and characteristics typical for *Escherichia dhe Salmonella* genres. All of 1.496 E. *coli* and 378 Salmonlla spp. isolates were obtained from poultries carrioned by colibacilliosis, salmonellosis or other infections with similar clinical signs of these infections.

IV. CONCLUSION

Clear identification and differentiation associated with the presence of commensally E. coli in the digestive tract of poultry is still a problem for the science of diagnostic laboratory of salmonellas and colibacilliosis in Albania. This was the main motive for undertaking this study and collecting data on the epidemiological situation in the poultry industry according to infections caused by APEC and Salmonella spp. The results of this study provided knowledge regarding the presence, distribution and behavior of E. *coli* and Salmonella spp., which are pathogenic for poultry, based on the use of innovative diagnostic methods. Although attenuated and live vaccines are continuously distributed for immunization of poultry against *enterobacterias*, *salmonellosis* and *colibacillosis*, these diseases remain among the most encountered bacterial infections in Albanian poultry industry. On the other hand, the all of 1.496 E. *coli* and 378 Salmonlla spp. strains, served as database for further analysing, regarding with their serotypisation and antibiotico resistance.These strains, will serve as database for further analysing, regarding with their serotypisation and antibiotico resistance.

ACKNOWLEDGMENT

The author would like to thank Prof. Pranvera Çabeli and Prof. Jasemin Bejleri for their contribution and the technical input that improved the overall study results presented in this paper.

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